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STUDY OF ERYTHROCYTE LIPID PEROXIDATION AND DEFORMABILITY IN INDIVIDUALS WITH SICKLE CELL TRAIT

ANNUAL AND FINAL REPORT

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Tread Mill exercise did not have any significant effect on the lipid peroxidation potential and deformability of RBC in normal (AA) subjects. However, it caused an increase in the lipid peroxidation potential and a loss of deformability of RBC in sickle cell trait (AS) subjects. Increased RBC lipid peroxidation in AS during exercise was accompanied with an accumulation of excessive amounts of superoxide radicals (due to increase in superoxide dismutase activity) and less decomposition of these free radicals. Furthermore, the modulation of peroxide scavengers of RBC in AS during exercise was associated with an increase in the intracellular calcium concentration and an increase in the RBC membrane density. Thus the loss of deformability in AS during exercise may be related to the change in RBC membrane density.

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FOREWORD

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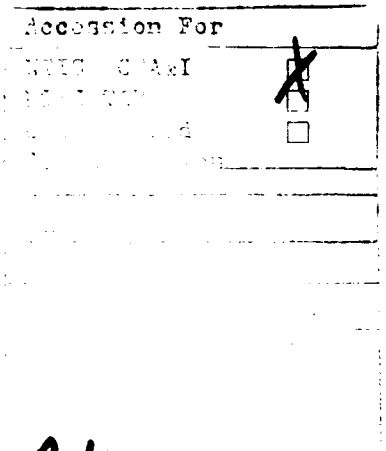

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1. INTRODUCTION

Controversy exists concerning the risks assumed by individuals with sickle cell trait (AS) while engaged in military activities that involve exposure to hypoxic environments and other stress situations (1-4).

Some claim that individuals with AS are relatively asymptomatic, have normal life span (5,6) and their responses to exercise are not impaired (6-12). Recently Weissman et al. (9-11) and Dillard et al (12) concluded that acute strenuous exercise does not have any significant effect on the cardiopulmonary and gas exchange responses of AS subjects.

However, there are numerous reports of unexpected "sudden death" occuring in subjects with heterozygous sickle cell trait (13-21). Most of these cases have been associated with high altitude and extreme exertions (13,14,18,22,23). Complications include renal failure (24,25), splenic infarction (26-32), intravascular coagulation (18,24,25), rhabdomyolysis and myoglobinuria (18,24,25,30,31), sicklemia (18), lowering of exercise values for heart rate and work load (33). York and Brierre found sickling in blood samples taken after 6 months of exposure of AS subjects to 10,000 feet levels in altitude chamber (34).

Kark et al (15) has recently described a 28 % increase in sudden unexplained deaths among black military recruits with AS compared with black military recruits without the trait. The risk of dying for black recruits with sickle cell trait were estimated at 1/3,200. Virtually all of the unexplained deaths occurred during the strenuous physical exertion and exercise associated with the 6 weeks of basic training, a time when many recruits could be presumed to be physically unconditioned. The risk for sudden, unexplained death among recruits appeared to increase with advancing age between 17 and 30 years of age.

Though the number of army recruits with homozygous sickle cell disease is probably small, eleven per cent of black recruits or about two to three per cent of the entire army may be potentially at risk for complications related to the heterozygous sickle cell trait (35). Systematic investigation into the pathophysiological and molecular mechanisms of these complications (related to sickling) are potentially very useful.

It is known that high morbidity and mortality in individuals with sickle cell disease are associated with RBC abnormality in terms of hemoglobin polymerization and membrane property changes (36). We have earlier demonstrated low levels of glutathione peroxidase and catalase activities in sickle red blood cells compared to normal erythrocytes (37). Further findings in SS RBC

membranes of fluorescent lipid pigments, malonaldehyde, membrane bound Heinz bodies, and reduced membrane lipid and unsaturated fatty acids suggest that membrane lipid peroxidation occurs (37,38). It is therefore important that we know whether the adverse effects of physical activity on AS individuals is related to RBC membrane changes.

2. OBJECTIVE

The objective of the current project funded by the Department of Army was to investigate whether the adverse effects of physical stress in AS individuals are associated with any biochemical change in RBC similar to those seen in SS. If such changes can be shown, they may be important in the production of clinical symptoms in the AS persons when they are under physically stressful conditions.

3. METHODOLOGY

18-40 yrs old male subjects with or without AS were subjected to continuous graded exercise on a Tread Mill according to Bruce protocol, with electrocardiographic monitoring, followed by 15 minutes of rest. Blood was drawn before exercise, immediately after exercise, and after 15 minutes of rest, for analysis of clinical, biochemical and physical parameters. In this project, so far we recruited 17 AA , 15 AS and 3 SS male subjects. SS subjects were not exposed to the Tread Mill exercise and their blood was used only for standardization of several experimental conditions. Data were treated statistically and the difference between AA and AS groups was determined by calculating the t statistic for two means, i.e., the nonpaired t-test (39). Differences at $p < 0.05$ were considered significant.

For clinical parameters, we recorded CBC and blood gas profile as well as cardiac function (EKG, blood pressure, heart rate). For biochemical parameters, we recorded (a) the protein content of RBC and ghosts, (b) the peroxide scavenger status of RBC by measuring the activity of SOD, GSH-Px and catalase, (c) the activity of NADPH generating enzymes, such as glucose 6-phosphate (G-6-P) and 6-phosphogluconate (6-PGA) dehydrogenase, (d) the activity of Na^+, K^+ -, and Ca^{++} - ATPases, (e) the intracellular and membrane Ca^{++} concentration, and (f) the peroxidation potential of RBC lipids. For physical parameters, we recorded the filtrability and deformability of RBC under oxygenated and deoxygenated conditions. These parameters were monitored by following the procedures referred in Table 1.

In this study, we prepared two types of RBC membranes differing in density and they are referred as heavy and light. Since the preparation of membrane is not parallel to current scientific technique, a schematic representation of the membrane preparation is shown in Fig. 1. In brief, AA and AS subjects were identified by electrophoresis of their blood specimens (40).

RBC was isolated from heparinized blood by conventional method and hemolyzed with 10 mM phosphate buffer, pH 7.4 and subjected to differential centrifugation and/or 35-50% linear sucrose density gradient (41). After centrifugation of the hemolyzate at 3,000 rpm for 10 min, the supernatant was centrifuged at 9,000 rpm for 15 min in a refrigerated Sorvall RC-5 centrifuge using a SS-34 rotor to obtain the heavy membrane pellet (HM). The supernatant containing loosely bound pellet was further centrifuged at 30,000 rpm for 30 min in a Beckman L8-M ultracentrifuge using a 70 Ti rotor to obtain the light membrane pellet (LM).

4. SIGNIFICANT FINDINGS

A. Effects of Physical Stress on Complete Blood Cell Count and Blood Gas Profile of Normal And Sickle Cell Trait Individuals.

The following important observations were made from data shown in Table 2.

1. The basal WBC count was significantly less in AS than AA. The WBC count was significantly increased during exercise and returned to the basal level after resting in both AA and AS.

2. The basal level of % O_2 Hb was significantly less in AS than AA. Exercise caused a significant increase in the level of % O_2 Hb in both. During resting, it started to decline towards the basal level in AA, however in AS, it continued to increase.

3. The basal level of % CO Hb level was significantly less in AS than AA. Exercise did not have any significant effect on the % CO Hb level in either AA or AS.

4. The basal level of % met Hb was significantly less in AS than AA. Exercise did not have any significant effect on % met Hb in AA. However in AS, it caused a significant increase.

5. The basal value of O_2 ct was same in AA and AS. Exercise caused a significant increase in O_2 content in both AA and AS. After resting, the value returns to the basal level in AA, but not in AS.

6. The basal pH value was significantly lower in AS than AA. Exercise caused a decrease in the pH value for both AA and AS. During resting, the pH started to increase towards the basal level in both.

7. The basal level of pO_2 was same in both AA and AS. Even though, exercise caused a significant increase in pO_2

in both AA and AS, it caused a significantly higher increase in AA than AS. However, while during resting, the pO_2 value returned to the basal level in AA, it did not return to the basal level in AS.

8. The basal value for BE (base excess) was not significantly different between AA and AS. The BE value was decreased from a positive to a negative number in AA due to exercise. After resting, this value started to increase towards the basal level in AA. In AS, the value was also decreased during exercise, but the change is minimal in comparison to that in AA.

9. The basal values of other parameters (RBC count, Hb, Hct %, mcv, pCO_2, HCO_3^- and CO_2 ct) were not significantly different between AA and AS. Furthermore, exercise did not have any significant effect on these parameters in either AA or AS.

B. Effects of Physical Stress on the density of RBC membrane of Normal and Sickle Cell Trait Individuals.

Physical stress causes an increase in the density of RBC membrane in AS but not in AA. This observation is based on the data obtained on the protein distribution between unhemolyzed cells, heavy and light membrane (Table 3, Fig. 2A). While exercise did not change the percent distribution of protein among these fractions in AA, exercise caused an increase in the amount of heavy membrane and hence protein, and a corresponding decrease in the amount of light membrane and hence protein in this fraction of AS (42-46).

C. Effects of Physical Stress on Peroxide Scavengers in Normal and Sickle Cell Trait RBC.

We did not observe any difference in the basal activity of catalase between AA and AS RBC. However, the basal activities of other peroxide scavengers (SOD and GSH-Px) were lower in AS than AA. While exercise did not affect the activity of either catalase or GSH-Px, it caused a remarkable increase in the activity of SOD in AS (Table 4, Fig. 3 A,B,C). Even though exercise did not have any significant effect on the activity of SOD in AA, it caused a significant increase in the activity of both catalase and GSH-Px.

Exercise did not have any effect on the lipid peroxidation potential of RBC in AA, but it caused an increase in the lipid peroxidation potential of RBC in AS (Table 5, Fig. 3 D). Increased lipid peroxidation potential in AS RBC during exercise may thus be due to the formation of excessive amount of superoxide radicals and less utilization of these free radicals. The increase in the ratio of SOD/ catalase

or GSH-Px in AS during exercise may thus result in the accumulation of H_2O_2 causing increased lipid peroxidation and membrane damage. It is possible that the change in RBC membrane density of AS individuals during exercise is related to the modulation of peroxide scavenger status of RBC (42-46).

D. Effects of Physical Stress on the activities of Na^+, K^+ - and Ca^{++} -ATPases and Ca^{++} Content of Normal and Sickle Cell Trait RBC.

Basal level of calcium in RBC was higher in AS than AA. It is important to note that the difference in the basal level of calcium in RBC between AA and AS is due to difference in the calcium content of the heavy membrane fraction between AA and AS. While exercise did not have any significant effect on calcium content in AA RBC, it caused a remarkable increase in AS, particularly in the membrane (Table 6, Fig. 2 C). Unhemolyzed cells accumulated more calcium during exercise in AS but not in AA. Thus, It is possible that increased accumulation of calcium in RBC may be responsible why RBC of AS individuals become more resistant to osmotic shock after exercise.

Basal activities of both Na^+, K^+ - and Ca^{++} - ATPases were similar in AA and AS for both heavy and light membranes (Table 7, Fig. 2 B and 2 D). Light membrane contained higher activity of both enzymes in both AA and AS. Exercise did not cause any remarkable change in the activity of any of the enzymes in either heavy or light membrane of AA. However in AS, exercise had a pronounced effect on the activity of these enzymes in the heavy membrane. For example, not only the activities of both enzymes were higher at peak exercise than the basal values in heavy membrane, there was a continuous increase in the activities of these enzymes during the fifteen minutes resting period. There was no significant effect of exercise on the activity of these enzymes in light membrane of AS.

Physical stress caused an increase in the density of RBC membrane in AS but not in AA. This observation has been based on the fact that while exercise did not change the percent distribution of protein among heavy and light membrane in AA, exercise caused an increase in the amount of heavy membrane protein, and a corresponding decrease in the amount of light membrane protein in AS (Table 3, Fig. 2 A). This change in membrane density may thus be due to the accumulation of calcium in AS as a result of the modulation of the membrane bound Na^+, K^+- and Ca^{++} - ATPases. It is possible that the change in membrane density and accumulation of calcium ion in RBC of AS individuals during exercise may cause loss of deformability and hence filtrability of RBC leading to intravascular coagulation

(44-46).

E. Effects of Physical Stress on Glucose 6 - Phosphate and 6 - Phosphogluconate Dehydrogenase Activity in Normal and Sickle Cell Trait RBC.

The basal activities of the NADPH generating enzymes were lower in AS than AA (Table 8). Exercise did not have any significant effect on the activity of glucose 6-phosphate dehydrogenase in either AA or AS. However, while exercise caused an increase in the activity of 6-PGA dehydrogenase in RBC of AA, it caused a decrease in the activity of this enzyme in RBC of AS. This observation may also explain why we observed a difference in the lipid peroxidation potential between RBC of AA and AS during physical exercise (Table 5, Fig. 3 D) (42-46).

F. Effects of Physical Stress on Intracellular Ca^{++} Concentration and Deformability of RBC in Normal and Sickle Cell Trait Individuals.

The objective of this study was to find out whether there is any association between intracellular Ca^{++} accumulation and loss of deformability of RBC in AA and AS individuals during exercise.

Fresh blood samples were obtained from 13 AA and 11 SCT subjects at three periods (before exercise, at peak exercise and after 15 minutes of resting). EDTA (2 mg/ml of blood) were used as anticoagulant. Red blood cells were washed 3 times with a buffer solution (118 mM NaCl, 5 mM KCl, 27 mM $NaHCO_3$, and 0.5 gm. per cent human serum albumin, pH 7.4) (47) under air-tight conditions. The washed cells were resuspended in both oxygenated (equilibrated with a gas mixture of 14 per cent O_2 , 5 per cent CO_2 and 81 per cent N_2) and deoxygenated (equilibrated with 0 per cent O_2 , 5 per cent CO_2 , and 95 per cent N_2) buffer solutions (4.1×10^5 cells / ml). pO_2, pCO_2 and pH of the suspensions were recorded in a blood gas analyzer (Model 213, Instrumentation Laboratories, Lexington, Mass) at 37^0 C, and also the cell count was recorded by a Coulter electronic particle counter (Model B, Coulter Electronics, Hialeah, Fla.) .

The red blood cell suspensions were filtered through polycarbonate sieves (Nucleopore corporation, Pleasanton, Calif.) (mean pore diameter, 5 µm, pore density, 4×10^5 pores per square centimeter). The sieve was placed in a filter holder (13 mm diameter) connected to two 30 ml glass syringes and a pressure transducer (Model P 23 XL Gould). The syringe was driven by an infusion pump (Model 351 Sage Instruments) at a constant flow rate (2 ml per minute). The pressure-time curve was monitored on a recorder (Model

79-4P-2 EEG/Polygraph, Grass Instrument, Quincy, Mass). At the start of each test, the cell-free buffer solution was pumped through the sieve, and a control pressure-time curve recorded before studying the pressure-time curve for RBC suspensions. The erythrocytes in the prefilter and postfilter fractions were counted by Coulter counter. Intracellular Ca^{++} concentration in the prefilter and postfilter fractions as well as in the cells retained by the microsieves were measured by atomic absorption spectroscopy (48).

Data in Table 9a and Fig. 4a and 4b show that exercise causes a loss of deformability of RBC in AS but not in AA. The status of RBC deformability was judged by monitoring the pressure change during filtration of RBC. There was an average increase in pressure of 40.3 mm Hg during filtration of RBC (4.1×10^5) cells under deoxygenated conditions (pO_2 = 20 mm Hg) through a 5 μm polycarbonate sieve. But in case of AA, there was only an increase in pressure of 10.5 mm Hg under the same condition. Furthermore, while there was a negligible change in pressure during filtration of AA RBC under oxygenated condition (pO_2 = 150-160 mm Hg), there was also an increase in pressure of 14.6 mm Hg for AS under the same condition.

The cells retained by the microsieve contained higher levels of calcium than those passing through the microsieve for both AA and AS. However, calcium concentration of the cells retained by the microsieve was significantly higher in AS than AA (Table 9b). Under both oxygenated and deoxygenated conditions, the concentration of calcium was significantly higher in all three fractions (prefiltered, postfiltered and unfiltered) in AS than AA even before exercise. Exercise did not have any significant effect on calcium ion concentration in AA, but it caused a significant increase in calcium ion concentration of all fractions in AS. Thus it is possible that the loss of deformability of RBC in AS during exercise may be related to the accumulation of calcium in RBC.

G. Heterogeneity of Human Red Blood Cell Membrane: Co-existence of Heavy and Light Membranes.

The erythrocyte membrane has long served as a convenient model system for the study of chemical and physical properties of cell membrane due to its relative simplicity. However, the chemical composition of RBC membrane may vary depending on the methods used for the isolation of the membrane. The most commonly used method of erythrocyte ghost preparation have employed hemolysis of RBC in hypotonic solutions for removal of hemoglobin. Even though this technique has been used, certain important variables, namely pH and ionic strength of the hemolyzing solutions

appear partially responsible for the conflicting reports on composition and function of RBC membrane. However, several investigators have previously encontered problems in preparing hemoglobin-free ghost. On centrifugation of the hemolyzed RBC, they observed two distinct pellets, namely a reddish pink translucent loose ghost pellet in "top" and an opaque red colored tightly packed ghost pellet at bottom.

However, these investigators failed to relate whether the difference in the physical nature of these pellets is due to the difference in the chemical composition. In this study, we provide evidence that human RBC membrane ghost is heterogeneous in nature and these two membranes (light and heavy) differ both in protein composition and biochemical properties.

RBC was isolated from heparinized human blood by conventional method and hemolyzed with 10 mM phosphate buffer, pH 7.4 and subjected to differential centrifugation and/or density gradient. Heavy membrane (HM) was pelleted down at 9000 rpm and the light membrane (LM) was collected at 30,000 rpm. A typical fractionation of RBC membrane on 35-50 % linear sucrose density gradient is shown in Fig. 5.

The amount of LM was double than that of HM per cell basis (Table 10). Even though there was no significant difference in the amount of calcium, the total activities of Na^+, K^+ - and Ca^{++} - ATPases, SOD, GSH-Px, catalase and both NADPH generating enzymes were significantly higher in LM than HM on per cell basis (Table 10) (41).

There was no significant difference in the specific activity of SOD, GSH-Px, catalase and NADPH generating enzymes between HM and LM. However, there was a significant difference in the specific activity of both the membrane bound ATPases between HM and LM (Table 11). For example, while the specific activity of Na^+, K^+- ATPase was higher in LM than HM, the specific activity of Ca^{++} -ATPase was higher in HM than LM.

SDS-solubilized HM and LM were electrophoresed in 10% SDS-polyacrylamide slab gels (49) in one dimension. The gels were stained with Coomassie blue R-250 and PAS (50) as well as with silver/Coomassie blue double stain (51) to detect membrane proteins (spectrin, actin, band 3, band 4.1 etc.), sialoglycoproteins and lipids on the gels. The quantity and molecular weight of the specific protein was determined by using both internal and external standards in a LKB 2222-010 UltroScan XL equipped with 2400 Gel Scan Software package.

There was a remarkable difference in the size and abundance of major polypeptides between these two membranes (Table

12, Fig. 6 and Fig. 7). For example, while component I was higher in LM than HM, HM contained higher level of component II than LM. Similarly, while band 4.1 was lacking in HM, there was a significant amount of this protein in LM. In general, in comparison to LM, HM contained more high molecular weight protein bands.

It is important to know whether there is any difference in the distribution pattern of protein in these two membrane fractions between AA and AS and whether exercise affects the structure and function of these membranes in either AA or AS.

H. Effects of Physical Stress on Peroxide Scavengers of RBC Membrane of Normal and Sickle Cell Trait Individuals.

Basal activity of catalase in either LM or HM was not different between AA and AS (Table 13). Basal activities of SOD and GSH-Px were significantly lower in AS than AA in both LM and HM. Exercise did not have any significant effect on the activities of any of the peroxide scavengers in either LM or HM in both AA and AS.

I. Effect of Physical Stress on Glucose 6-Phosphate and 6-Phosphogluconate Dehydrogenase Activity in RBC Membrane of Normal and Sickle Cell Trait Individuals.

Basal activities of the NADPH generating enzymes were significantly lower in AS than AA in LM but not in HM (Table 14). Exercise did not have any significant effect on the membranes in either AA or AS.

5. DISCUSSION

Physical stress has been thought to be hazardous to individuals with sickle cell trait (AS) and cause sudden death. The adverse effects of physical stress in AS individuals may be associated with biochemical changes in RBC. In this project, we have monitored the effects of Tread Mill Exercise on several biochemical parameters of RBC in 18-40 years old normal (AA) and and AS male subjects.

There was no significant difference in the basal activity of catalase in RBC between AA and AS; however the basal activities of other peroxide scavengers (superoxide dismutase, SOD and glutathione peroxidase, GSH-Px) were significantly lower in AS RBC than normal RBC. While exercise did not affect the activity of either catalase or GSH-Px, it caused a significant increase in the activity of SOD in RBC of AS. The increase in the ratio of SOD/ catalase or GSH-Px during physical stress may therefore be responsible for the observed increase in the lipid peroxidation potential of RBC in AS. However in RBC of AA, while exercise did not have any significant effect on the activity of SOD, it caused

a significant increase in the activity of both catalase and GSH-Px. As a consequence, there was no accumulation of H_2O_2 in RBC of AA during exercise.

The basal activities of the NADPH generating enzymes (glucose 6- phosphate dehydrogenase and 6- PGA dehydrogenase) in RBC were lower in AS than AA. Exercise did not have any significant effect on the activity of glucose 6-phosphate dehydrogenase, but it modulated the activity of 6-PGA dehydrogenase in both AA and AS. While exercise caused an increase in the activity of 6-PGA dehydrogenase in AA, it caused a decrease in the activity of this enzyme in AS RBC. This observation may also explain why we observed a difference in the lipid peroxidation potential of RBC between AA and AS due to exercise.

The exact chemical composition of RBC membrane may vary depending on the methods used for the isolation of membrane. Inspite of the general belief that human RBC membrane is homogeneous, we fractionated RBC membrane by differential centrifugation and/or density gradient into two distinct types, designated as "heavy" and "light". These membranes differ in several biochemical properties.

The amount of light membrane (LM) was double than that of heavy membrane (HM) per cell basis. Furthermore, while there was no significant difference in the calcium concentration and the activity of any of the NADPH generating enzymes (glucose 6-phosphate dehydrogenase and 6-PGA dehydrogenase) between HM and LM, the activities of several enzymes, such as, Na^+ , K^+ -ATPase, Ca^{++} -ATPase, SOD, GSH-Px and catalase were significantly higher in LM than HM.

The electrophoretic pattern on 10 % SDS-polyacrylamide reveals that in comparison to LM, HM contains more high mol. wt. protein bands. It is important to know whether there is any difference in the distribution pattern of protein in these two membrane fractions between AA and AS and whether exercise affects the structure and function of these membranes in either AA or AS.

Physical stress caused an increase in the density of RBC membrane in AS but not in AA. This observation was based on the protein distribution data between unhemolyzed cells, heavy and light membrane. While exercise did not change the percent distribution of protein among these fractions in AA, exercise caused an increase in the amount of heavy membrane and hence protein, and a corresponding decrease in the amount of light membrane and hence protein in this fraction of AS.

Basal level of calcium was not different in either unhemolyzed cells or LM between AA and AS. However, the basal level of calcium in RBC hemolyzate and HM was significantly higher in AS than AA. Exercise did not have any significant effect on the calcium content of any of the RBC fractions in AA. Exercise

caused a significant increase in the calcium content of all the fractions of RBC, particularly in HM and unhemolyzed cells. The accumulation of calcium in RBC membrane of AS during exercise may thus be related to the increase in membrane density.

Basal activity of any of the membrane bound ATPases was similar in either LM or HM of AA and AS. Exercise did not have any significant effect on the membrane bound ATPases in either LM or HM of AA. However in AS, eventhough exercise did not have any significant effect on any of the membrane bound ATPases in LM, it caused a significant increase in both Na^+, K^+- and Ca^{++} -ATPases in HM. The increased activity of the ATPases in HM may thus be related to the increased accumulation of calcium in RBC of AS during exercise.

The increase in RBC membrane density of AS during exercise may thus be due to (a) accumulation of calcium as a result of increased Na^+, K^+- and Ca^{++} -ATPases and (b) increase in the lipid peroxidation potential of RBC membrane.

It is possible that the change in membrane density in AS RBC during exercise may cause loss of deformability. The data collected in this laboratory have indicated that exercise does not have any remarkable effect on the filtrability (deformability) of AA RBC, but it causes a significant decrease in the filtration rate of AS RBC, similar to that seen in SS RBC. For example, there is an average increase in pressure of 40.3 mm Hg during filtration of RBC (4.1×10^5 cells) under deoxygenated conditions (pO_2 = 20 mm Hg) through a 5 µm polycarbonate sieve.

Thus, it is evident from these studies that physical stress causes modulation of some important biochemical properties of the RBC which may be responsible for the clinical abnormality seen in AS subjects during exercise.

6. CONCLUSIONS

Though homozygous sickle cell disease is very rare in army recruits, eleven per cent of Black recruits (2-3 % of the entire army) are potentially at risk for complications related to the heterozygous sickle cell trait (SCT) with microvascular complications frequently associated with high altitude and extreme exertions. Current studies in this laboratory suggest that physical stress causes (a) an increase in the lipid peroxidation potential of RBC membrane, (b) an increase in the density of RBC membrane, and (c) a decrease in the deformability of RBC of SCT individuals. It is possible that the change in the RBC membrane structure during exercise may be functionally related to the loss of deformability in SCT.

Vast majority of workers now agree that the biological selectivity occurs in the cell membrane and any alteration in the membrane structure will lead to a change in the permeability

characteristic of the cells and their function (52). It is known that lectins interact with specific sugar moieties located on the surface of a variety of cells (53). Major sialoglycoproteins of human erythrocytes have high affinity for a wide variety of lectins (54). It has been reported that the minor glycoprotein (band III and component a) of human erythrocytes is responsible for anion transport across the membrane and is the high affinity receptor for Concanavalin A (Con A) (55). If we find any change in the Con A binding characteristics of the erythrocytes due to exercise, it will indicate that there is a change in the composition of the surface glycoprotein and hence anion transport function.

Therefore, it is necessary to study the effects of physical stress on the distribution pattern of protein and glycoprotein in RBC membrane. Furthermore, in view of the evidence (56) that spectrin plays an important role in regulating the deformability characteristic of RBC, it is important to know whether exercise causes any change in the spectrin so that the deformability of RBC is affected. If we find any alteration in types of membrane proteins due to physical stress, we will attempt to isolate and characterize these proteins by proteolysis (57), treatment with neuraminidase (58), treatment with cold NaOH (59) and treatment with acidic chloroform/methanol, followed by gel electrophoresis.

Furthermore, since we have preliminary data (41) which indicate that HM contains more high molecular weight protein bands than LM in AA, it remains to be seen whether such difference also exists in AS and whether exercise increases these proteins in AS. Therefore, we will try to isolate these protein bands by preparative electrophoresis (60).

It is known that the depletion of cellular ATP causes a loss of deformability of RBC (61). Since the active transport of certain nutrients, such as sugars and fatty acids depend on cellular ATP, it is possible that physical stress may cause a disturbance in the nutrient transport of RBC.

Thus, it is important that we find out the mechanism by which physical stress causes a decrease in the RBC deformability of SCT. In order to achieve this goal, our future studies will focus on the effects of physical exercise on (a) membrane lipid and protein composition, (b) fluidity of membrane and RBC, (c) lectin binding properties of RBC membranes, and (d) nutrient transport properties of RBC and in both AA and SCT.

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TABLE 1

ASSAY SYSTEMS

Protein	Lowry et al., J. Biol. Chem. 193: 265, 1951.
SOD	Hyland et al., Anal. Biochem. 135: 280, 1983.
GSH-Px	Flohe and Gunzler, Methods in Enzymol. 105: 114, 1984.
Catalase	Sinha, Anal. Biochem. 47: 389, 1972.
G-6-P & 6-PGA DH	Beutler, in Methods in Hematol: Red Cell Metab. 16 : 57, 1986.
$Na^+ - K^+$ - and Ca++ -ATPases	Sen & Ray, Arch Biochem. Biophys. 198: 548, 1979.
Calcium	Olson, J. Membrane Biol. 48: 265, 1979.
Lipid Peroxidation Potential	Stocks & Dormandy, Br. J. Haematol. 20: 95, 1971.
Deformability of RBC	Usami et al., J. Lab. & Clin. Med. 86: 274, 1975.

TABLE 2

EFFECT OF PHYSICAL STRESS ON COMPLETE BLOOD COUNT (CBC) AND BLOOD GAS PROFILE OF NORMAL AND SICKLE CELL TRAIT INDIVIDUALS

Parameters	Before Exercise		At Peak Exercise		Rest	
	AA	AS	AA	AS	AA	AS
RBC ($\times 10^9$ /ml)	5.19 (0.06)	5.31 (0.06) d	5.30 (0.03) d	5.66 (0.11) a	5.11 (0.08)	5.54 (0.03)
WBC ($\times 10^9$ /ml)	5.07 (0.27)	4.15 (0.17)	7.53 (0.22)	6.43 (0.69)	5.17 (0.04)	4.77 (0.25)
Hgb (g/dl)	14.70 (0.85)	15.36 (0.29)	15.31 (0.16)	16.39 (0.39)	14.33 (0.18)	15.95 (0.26)
Hct (%)	43.71 (0.52)	45.50 (0.76)	45.68 (0.43)	48.38 (0.48)	44.20 (0.61)	46.60 (0.46)
mcv (fl)	86.37 (0.60)	86.00 (0.54) b	86.88 (0.60) d	85.83 (0.49) d	86.83 (0.86) a	85.80 (0.59) d
% O_2 Hb	50.46 (1.93)	44.00 (0.92) d	70.03 (2.57)	59.30 (0.85)	60.00 (1.59)	70.25 (1.20)
% CO Hb	2.45 (0.32)	0.80 (0.20) d	1.82 (0.28)	0.85 (0.10) d	2.36 (0.30)	0.95 (0.11) d
% met Hb	0.33 (0.03)	0.11 (0.01)	0.25 (0.02) a	0.15 (0.01) d	0.44 (0.05)	0.35 (0.02) d
O_2 ct	9.46 (0.66)	9.20 (0.48) d	12.92 (0.65) d	13.05 (0.49) d	10.90 (0.51) d	14.85 (0.48)
pH	7.339 (0.004)	7.308 (0.005)	7.227 (0.007)	7.247 (0.008)	7.295 (0.006)	7.296 (0.009)
p CO_2 (mm Hg)	46.67 (2.86)	55.22 (3.13)	50.45 (1.48) d	52.35 (0.04) c	46.44 (1.29)	53.48 (1.42) d
p O_2 (mm Hg)	32.62 (1.32)	30.91 (0.76)	51.88 (2.58) d	33.65 (2.72)	33.0 (0.81) d	36.65 (2.84)
BE (mEQ/L)	6.24 (0.41)	2.85 (1.84)	- 4.70 (0.48)	1.40 (0.70)	0.74 (0.37)	2.02 (1.04)
HCO_3^- (mEQ/L)	27.68 (0.33)	27.41 (0.16)	19.57 (0.42)	24.95 (0.88)	22.56 (0.68)	24.45 (1.56)
CO_2 ct (mEQ/L)	29.21 (0.33)	29.12 (0.16)	21.01 (0.44)	26.55 (0.31)	24.00 (0.72)	25.97 (0.62)

LEGENDS

Table 2. Basal levels of WBC, % O_2 Hb, % CO Hb, % met Hb and pH were significantly lower in AS than AA (for b, $p = <0.02$ and for d, $p = <0.001$). The basal levels of other parameters were not significantly different between AA and AS. Exercise caused a significant increase in WBC, % O_2 Hb, O_2 ct and pO_2 in both AA and AS (for a, $p = <0.01$, for c, $p = <0.05$ and for d, $p = <0.001$). During resting these values approached towards the basal levels in AA. In AS, except WBC, other parameters (% O_2 Hb, O_2 ct and pO_2) continued to increase. While exercise did not have any significant effect on % met Hb in AA, it caused a significant increase of % met Hb in AS. While exercise caused a significant decrease in pH for both AA and AS, it caused only a significant decrease in BE for only AA and not AS (for d, $p = <0.001$).

TABLE 3

EFFECT OF PHYSICAL STRESS ON PROTEIN CONTENT OF RBC AND GHOST OF NORMAL (AA) AND SICKLE CELL TRAIT (AS) INDIVIDUALS

Sample	Before Exercise		At Peak Exercise		Rest	
	AA	AS	AA	AS	AA	AS
RBC Hemolysate	43.82 (2.61)	44.02 (1.95)	44.12 (1.88)	40.23 (1.96)	42.60 (2.90)	42.86 (2.19)
Unhemolyzed Cells	0.19 (0.03)	0.16 (0.02)	0.14 (0.02)	0.26 (0.05)	0.17 (0.03)	0.21 (0.04)
Heavy Membrane	0.39 (0.04)	0.48 (0.03)	0.41 (0.05)	0.68[b] (0.06)	0.46 (0.05)	0.65[a] (0.04)
Light Membrane	0.90 (0.08)	0.88 (0.10)	0.82 (0.05)	0.60[b] (0.03)	0.87 (0.09)	0.62[c] (0.05)
Plasma	107.53 (4.28)	104.37 (12.81)	111.60 (1.27)	106.45 (6.88)	101.59 (9.06)	102.05 (5.62)

Values for each sample except plasma are mean pg / cell. Values for plasma are mean mg / ml. Number in parenthesis represents S.E.M. of 10 samples. Values for HM and LM in AS after exercise and resting are significantly different from those before exercise (for a, $p = <0.01$; for b, $p = <0.02$; and for c, $p = <0.05$). Exercise causes an increase in the amount of HM protein and a decrease in the amount of LM protein in AS.

TABLE 4

EFFECT OF PHYSICAL STRESS ON PEROXIDE SCAVENGERS OF RBC OF NORMAL (AA) AND SICKLE CELL TRAIT (AS) INDIVIDUALS

Parameters	Before Exercise		At Peak Exercise		Rest	
	AA	AS	AA	AS	AA	AS
A. SOD (unit x 10^{-9} / cell)						
	68.50 (9.65)	44.14[c] (5.34)	81.99 (6.16)	71.81[c] (10.17)	69.96 (3.18)	83.94[c] (12.97)
B. GSH-Px (μ moles x 10^{-10} NADPH oxidised /cell / min)						
	29.48 (2.26)	16.60[b] (2.29)	35.50[c] (0.04)	17.58 (3.16)	36.05[c] (2.43)	18.92 (3.64)
C. Catalase (μ moles x 10^{-7} of H_2O_2 decomposed / cell / min)						
	27.05 (1.81)	26.77 (1.49)	32.77[c] (1.28)	28.11 (1.29)	29.67 (1.89)	25.12 (1.49)

Number in parenthesis represents S.E.M. of 10 samples. Basal activity of catalase was not different between AA and AS. Basal activities of SOD and GSH-Px were significantly lower in AS than AA (for b, $p = <0.02$ and for c, $p = <0.05$). Exercise did not have any effect on the activities of GSH-Px and catalase, but significantly increased the SOD activity in AS (for c, $p = <0.05$). Exercise caused a significant increase in the activities of GSH-Px and catalase in AA (for c, $p = <0.05$), but it did not have any significant effect on the SOD activity in AA.

TABLE 5

EFFECT OF PHYSICAL STRESS ON IN VITRO LIPID PEROXIDATION OF RBC OF NORMAL (AA) AND SICKLE CELL TRAIT (AS) INDIVIDUALS.

Sample	Before Exercise		At Peak Exercise		Rest	
	AA	AS	AA	AS	AA	AS
RBC	16.21 (3.20)	18.91 (2.70)	19.98 (3.79)	28.73[b] (2.27)	14.11 (2.15)	21.37 (4.77)
RBC + H_2O_2	35.72 (3.20)	37.21 (2.03)	34.30 (5.47)	44.05[c] (1.73)	38.47 (5.07)	40.63 (5.32)
RBC + NaN	24.93 (3.68)	26.67 (1.87)	20.78 (3.83)	32.12 (2.52)	15.20 (3.34)	24.49 (2.12)
RBC + NaN + H_2O_2	444.69 (28.17)	528.83 (21.65)	382.99 (30.73)	612.58[c] (22.17)	446.68 (32.15)	560.61 (25.46)

Values are mean μ moles x 10^{-9} MDA / cell. Number in parenthesis represents S.E.M of 10 samples. Exercise does not have any effect on the lipid peroxidation potential of RBC in AA, but it causes a significant increase in the peroxidation potential in RBC of AS (for b, $p = <0.02$ and for c, $p = <0.05$.

TABLE 6

EFFECT OF PHYSICAL STRESS ON CALCIUM CONTENT OF RBC AND GHOST OF NORMAL (AA) AND SICKLE CELL TRAIT (AS) INDIVIDUALS

Sample	Before Exercise		At Peak Exercise		Rest	
	AA	AS	AA	AS	AA	AS
RBC Hemolysate	24.58 (3.80)	67.99[d] (7.99)	36.43 (1.79)	94.18[c] (6.96)	26.09 (3.30)	78.78 (10.70)
Unhemolyzed Cells	3.18 (0.62)	5.61 (1.64)	4.90 (1.53)	14.83[a] (1.91)	3.46 (1.14)	12.02 (3.12)
Heavy Membrane	5.48 (1.26)	14.86[c] (1.53)	5.13 (1.09)	24.21[a] (2.02)	4.34 (1.51)	18.32 (1.92)
Light Membrane	12.85 (3.97)	13.18 (2.47)	8.08 (2.04)	23.97[c] (3.73)	9.64 (1.46)	23.92[c] (3.23)

Values are mean f moles x 10^{-9} /cell. Number in parenthesis represents S.E.M. of 10 samples. Basal level of calcium was not different in unhemolyzed cells and light membrane between AA and AS. Basal level of calcium was significantly higher in RBC hemolysate and heavy membrane in AS than AA (for c, $p = <0.05$ and for d, $p = <0.001$. Exercise did not have any significant effect on calcium level in any of the RBC fractions of AA. However, it caused a significant increase in calcium content of RBC hemolysate, unhemolyzed cells, light membrane and heavy membrane of AS (for a, $p = <0.01$ and for c, $p = <0.05$).

TABLE 7

EFFECT OF PHYSICAL STRESS ON Na^+, K^+ - AND Ca^{++} - ATPase ACTIVITIES IN RBC MEMBRANES OF NORMAL AND SICKLE CELL TRAIT INDIVIDUALS

Sample	Before Exercise		At Peak Exercise		Rest	
	AA	AS	AA	AS	AA	AS
A. Na^+, K^+ - ATPase						
Heavy Membrane	41.60 (7.90)	36.70 (6.96)	30.28 (3.60)	55.86 (12.72)	43.81 (5.62)	97.37[c] (22.59)
Light Membrane	91.48 (7.32)	79.20 (18.63)	84.18 (5.83)	94.92 (8.74)	82.10 (6.70)	87.75 (14.63)
B. Ca^{++} - ATPase						
Heavy Membrane	66.22 (9.68)	58.22 (9.31)	52.37 (6.97)	108.64[b] (14.43)	58.76 (10.12)	149.32[d] (17.24)
Light Membrane	97.79 (8.17)	75.74 (11.01)	84.62 (19.16)	102.86 (17.99)	94.93 (11.89)	103.05 (22.39)

Values are mean n moles x 10^{-9} P_i formed / cell / hr. Number in Parenthesis represents S.E.M. of 10 samples. Basal levels of both ATPases were same in AA and AS for both LM and HM. Exercise has no significant effect on either LM or HM in AA. However in AS, even though exercise did not have any significant effect on LM, it causes a significant increase in the activity of both ATPases in HM (for b, $p = <0.02$, for c, $p = <0.05$ and for d, $p = <0.001$.

TABLE 8

EFFECT OF PHYSICAL STRESS ON GLUCOSE-6-PHOSPHATE AND 6-PHOSPHOGLUCONATE DEHYDROGENASE ACTIVITY IN RBC OF NORMAL (AA) AND SICKLE CELL TRAIT (AS) INDIVIDUALS

Parameters	Before Exercise		At Peak Exercise		Rest	
	AA	AS	AA	AS	AA	AS
A. Glucose -6- Phosphate Dehydrogenase						
	136.91 (12.61)	23.60[d] (6.50)	150.18 (6.85)	20.81 (0.20)	137.35 (7.59)	26.12 (3.40)
B. 6- Phosphogluconate Dehydrogenase						
	10o.31 (5.72)	26.84[d] (3.09)	133.07[b] (7.02)	16.54[c] (2.23)	95.27 (5.81)	20.12 (4.89)

Values are mean μ moles x 10^{-9} NADP reduced /cell / min. Number in parenthesis represents S.E.M. of 10 samples. Basal activities of G-6-P dehydrogenase and 6-PGA dehydrogenase are significantly lower in AS than AA (for d, $p = <0.001$). Exercise caused a significant increase in the activity of 6-PGA dehydrogenase in AA (for b, $p = <0.02$), whereas it causes a significant decrease in the activity of this enzyme in AS (for c, $p = <0.05$).

TABLE - 9a

EFFECT OF TREAD MILL EXERCISE ON DEFORMABILITY OF NORMAL AND SICKLE CELL TRAIT RBC

	BEFORE EXERCISE		AT PEAK EXERCISE		REST	
PARAMETER	AA	AS	AA	AS	AA	AS
Oxygenated RBC	14.00 (3.07)	20.57 (2.73)	16.66 (4.73)	35.14 a (2.65)	18.50 (4.20)	36.43 c (6.05)
Deoxygenated RBC	19.00 (2.86)	35.57 a (4.20)	30.50 b (2.38)	75.86 a (8.73)	28.00 b (3.52)	71.14 a (9.83)

Values are mean pressure rise in mm Hg during filtration of RBC. Number in parenthesis represents S.E.M. of 10 samples. Under oxygenated condition basal deformability status of RBC was not significantly different between AA and AS. However, under deoxygenated condition, basal deformability was lower in AS than that in AA (for a, $p = <0.01$). Exercise did not cause any change in deformability in AA under oxygenated condition, however causes a significant loss under deoxygenated condition (for b, $p = < 0.02$). Exercise caused a significant loss of deformability of RBC in AS both under oxygenated and deoxygenate conditions (for a, $p = < 0.01$, and for c, $p = < 0.05$). Loss of deformability due to exercise was significantly higher in AS than AA under both oxygenated and deoxygenated condition.

TABLE - 9b

INTRACELLULAR CONCENTRATION OF CALCIUM ION IN PREFILTERED, PPOSTFILTERED AND UNFILTERED RBC OF NORMAL (AA) AND SICKLE CELL TRAIT (AS) IN OXYGENATED AND DEOXYGENATED CONDITION : EFFECT OF EXERCISE.

	BEFORE EXERCISE		AT PEAK EXERCISE		REST	
PARAMETER	AA	AS	AA	AS	AA	AS
Oxygenated						
Prefiltered	21.68 (3.08)	46.76[d] (2.19)	27.75 (2.64)	67.97[d] (2.17)	24.03 (2.72)	62.18[a] (2.75)
Postfiltered	19.68 (1.62)	41.30[d] (2.07)	21.83 (3.55)	55.28[a] (2.81)	20.32 (1.46)	56.10[a] (2.08)
Unfiltered	32.16 (1.64)	62.58[d] (1.10)	34.79 (1.82)	101.20[d] (2.19)	32.74 (1.82)	80.14[d] (1.98)
Deoxygenated						
Prefiltered	23.18 (3.51)	56.79[d] (3.15)	31.64 (3.07)	90.62[d] (2.53)	26.92 (2.14)	67.87[d] (2.50)
Postfiltered	21.19 (1.70)	47.06[d] (2.01)	27.61 (1.62)	59.47[a] (2.79)	22.78 (1.57)	59.40[a] (2.50)
Unfiltered	36.82 (4.11)	80.95[d] (4.02)	38.68 (2.46)	128.87[d] (2.46)	36.27 (1.73)	99.67[a] (3.25)

Values are mean f moles x 10^{-9} / cell. Number in parenthesis represents S.E.M. of 10 samples. Under both oxygenated and deoxygenated conditions, the concentration of calcium was significantly higher in all fractions of AS than AA even before exercise (for a, $p = < 0.001$). Exercise did not have any significant effect on AA, but it caused a significant increase in calcium concentration of all fractions in AS (for d, $p = <0.001$, for a, $p = <0.01$).

TABLE 10

Comparison of the Distribution of Some Important Biochemical Parameters between Heavy and Light Membranes of Normal Human Red Blood Cells.

Parameters	HM	LM
Protein (pg)	0.39 ± 0.04	0.90 ± 0.08[d]
Calcium (fmol x 10^{-9})	5.48 ± 1.26	12.85 ± 3.97
Na^+, K^+ - ATPase (nmol P_i x 10^{-9} /hr)	41.60 ± 7.90	91.48 ± 7.32[d]
Ca^{++} - ATPase (nmol P_i x 10^{-9}/hr)	66.22 ± 9.68	97.79 ± 8.19[c]
SOD (units x 10^{-9})	2.31 ± 0.49	4.23 ± 0.25[d]
GSH-Px (umol x 10^{-10} NADPH oxidized / min)	0.50 ± 0.05	0.67 ± 0.05[c]
Catalase (umol x 10^{-7} H_2O_2 decomposed / min)	0.12 ± 0.01	0.41 ± 0.06[d]
G-6-P dehydrogenase (umol x 10^{-9} NADP reduced / min)	2.27 ± 0.28	3.53 ± 0.23[a]
6-PGA dehydrogenase (umol x 10^{-9} NADP reduced / min)	1.03 ± 0.30	3.06 ± 0.22[d]

Results are expressed as mean + S.E. of 10 samples on per cell basis. Protein content and activities of Na^+, K^+ - ATPase, Ca^{++} - ATPase, SOD, GSH-Px and catalase are significantly higher in LM than HM (for c, $p = <0.05$ and for d, $p = <0.001$. No significant difference in calcium content and activities of the NADPH generating enzymes was observed between LM and HM.

TABLE 11

Comparison of the Specific Activity of Some Important Enzymes Between Heavy and Light Membranes of Human RBC

Parameters	HM	LM
Hemoglobin (ng)	0.12 ± 0.02	0.21 ± 0.03[b]
Na^+, K^+ - ATPase (nmol P_i/hr)	75.35 ± 7.18	118.76 ± 8.32[a]
Ca^{++} - ATPase (nmol P_i/hr)	172.12 ± 11.70	108.08 ± 7.38[d]
SOD (units)	3.32 ± 0.89	4.92 ± 0.79
GSH-Px (µmol NADPH oxidized/ min)	0.10 ± 0.02	0.07 ± 0.01
Catalase (µmol H_2O_2 decomposed / min)	3.38 ± 0.33	3.24 ± 0.49
G-6-P dehydrogenase (µmol NADP reduced / min)	7.59 ± 1.95	3.96 ± 0.30
6-PGA dehydrogenase (µmol NADP reduced / min)	2.97 ± 0.64	2.78 ± 0.85

Results are expressed mean ± S.E. of 10 samples on the basis of per mg protein. There was no significant difference in the specific activity of SOD, GSH-PX, catalase and NADPH generating enzymes between LM and HM. However, there was a significant difference in the specific activities of Na^+, K^+ - and Ca^{++} - ATPases between LM and HM (for a, $p = <0.01$; for b, $p = <0.02$ anf for d, $p = <0.001$). While the specific activity of Na^+, K^+-ATPase was higher in LM than HM, the specific activity of Ca^{++} -ATPase was higher in HM than LM.

TABLE 12

Distribution of Major Ghost Polypeptides Between Heavy and Light Membranes of Human RBC

Band	Identity (Tentative)	Mol. Wt.	Relative Abundance	
			Heavy	Light
1	Component I	~ 250,000	1.53	19.53
2	Component II	~ 250,000	23.11	9.88
3			5.58	5.72
3a			6.54	-
4			8.68	4.09
5	Component III	~ 90,000	10.81	14.57
5a		~ 88,000	3.25	-
6			9.27	3.02
7	Band 4.1	~ 72,000	-	7.52
8	Band 4.2	~ 66,000	4.15	4.59
8a			7.62	-
9			1.54	3.58
10			5.07	1.81
11			1.92	1.66
11a			1.62	-
12			-	2.53
12a			1.83	-
13-20			7.47	21.92

Approximate molecular weight of each protein band was measured by calibration of the gels with mixtures of molecular weight markers. Some of the major polypeptides was tentatively identified by comparing with the reported values of major ghost polypeptides (Fairbanks, G., Steck, T. L. and Wallach, D. F. H., Biochem. 10 : 2606-2617, 1971). Values are mean of 10 samples.

TABLE 13

EFFECT OF PHYSICAL STRESS ON PEROXIDE SCAVENGERS OF RBC MEMBRANE OF NORMAL (AA) AND SICKLE CELL TRAIT (AS) INDIVIDUALS

Sample	Before Exercise		At Peak Exercise		Rest	
	AA	AS	AA	AS	AA	AS
A. SOD (unit x 10^{-9} / cell)						
Heavy Membrane	2.31 (0.29)	1.25[a] (0.13)	2.54 (0.54)	1.66 (0.43)	1.84 (0.39)	1.61 (0.47)
Light Membrane	4.23 (0.75)	1.67[a] (0.31)	3.96 (0.65)	1.75 (0.31)	2.72 (0.73)	1.90 (0.36)
B. GSH-Px (μ moles x 10^{-10} NADPH oxidised /cell / min)						
Heavy Membrane	0.50 (0.05)	0.19[d] (0.04)	0.33 (0.04)	0.43 (0.05)	0.49 (0.02)	0.30 (0.07)
Light Membrane	0.67 (0.05)	0.24[d] (0.04)	0.47 (0.05)	0.23 (0.02)	0.46 (0.06)	0.38 (0.02)
C. Catalase (μ moles x 10^{-7} of H_2O_2 decomposed / cell / min)						
Heavy Membrane	0.12 (0.03	0.14 (0.02)	0.15 (0.02)	0.20 (0.03)	0.12 (0.02)	0.28 (0.07)
Light Membrane	0.41 (0.01)	0.39 (0.02)	0.49 (0.03)	0.30 (0.04)	0.38 (0.01)	0.20 (0.03)

Number in parenthesis represents S.E.M. of 10 samples. Basal activity of catalase in either LM or HM was not different between AA and AS. Basal activities of SOD and GSH-Px are significantly lower in AS than AA in both LM and HM (for a, $p = <0.01$ and for d, $p = <0.001$). Exercise did not have any significant effect on the activities of any of the peroxide scavengers in either LM or HM in both AA and AS.

TABLE 14

EFFECT OF PHYSICAL STRESS ON GLUCOSE-6-PHOSPHATE AND 6-PHOSPHOGLUCONATE DEHYDROGENASE ACTIVITY IN RBC MEMBRANE OF NORMAL (AA) AND SICKLE CELL TRAIT (AS) INDIVIDUALS

Sample	Before Exercise		At Peak Exercise		Rest	
	AA	AS	AA	AS	AA	AS
A. Glucose -6- Phosphate Dehydrogenase						
Heavy Membrane	2.27 (0.38)	1.38 (0.49)	1.20 (0.30)	0.88 (0.39)	1.48 (0.14)	0.45 (0.22)
Light Membrane	3.53 (0.73)	0.71[a] (0.18)	3.10 (0.83)	0.74 (0.29)	1.62 (0.11)	0.46 (0.15)
B. 6- Phosphogluconate Dehydrogenase						
Heavy Membrane	1.03 (0.30)	0.47 (0.12)	0.96 (0.23)	0.51 (0.19)	0.83 (0.16)	0.51 (0.27)
Light Membrane	3.06 (0.82)	0.81[c] (0.30)	3.12 (0.95)	0.55 (0.24)	1.17 (0.24)	0.26 (0.03)

Values are mean μ moles x 10^{-9} NADP reduced /cell / min. Number in parenthesis represents S.E.M. of 10 samples. Basal activities of G-6-P dehydrogenase and 6-PGA dehydrogenase are significantly lower in AS than AA in LM, but not in HM (for a, $p = <0.01$) and for c, $p = <0.05$). Exercise did not have any significant effect on the membranes in both AA and AS.

Preparation of Erythrocyte Membrane

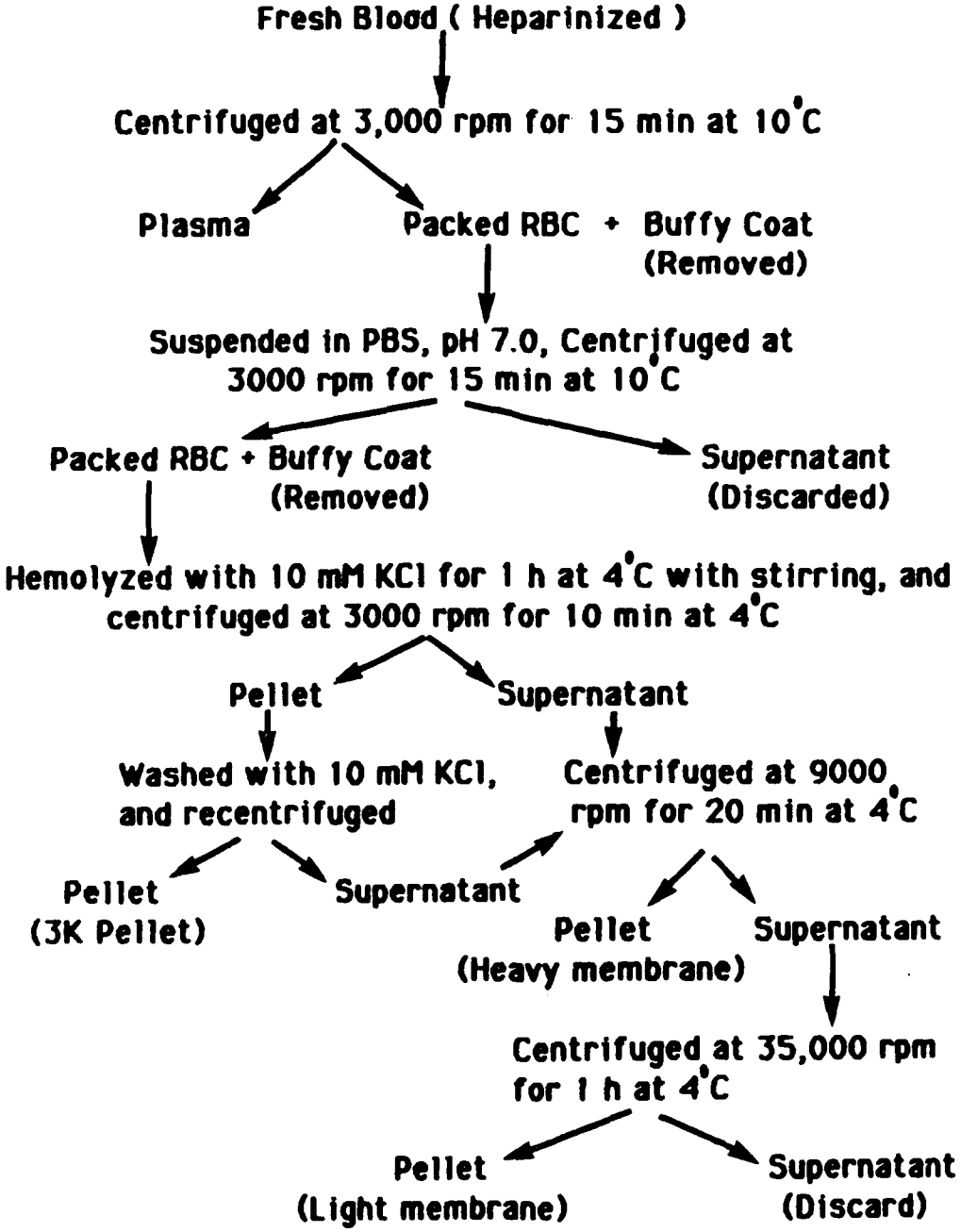

Fig. 1

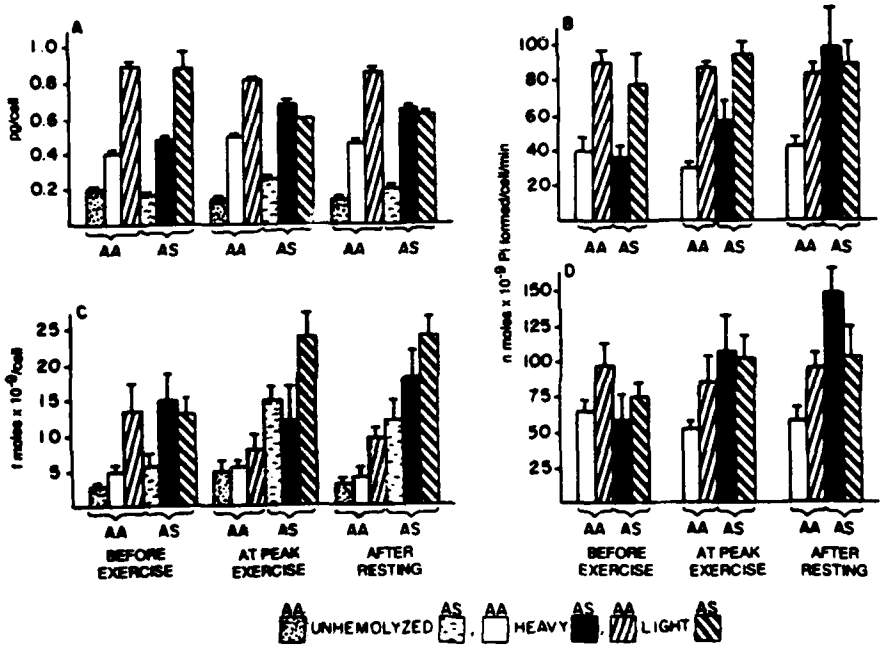

Fig- 2

EFFECT OF PHYSICAL STRESS ON (A) PROTEIN CONTENT, (B) Na^+, K^+ - ATPase, (C) Ca^{++} - ION CONTENT AND (D) Ca^{++} - ATPase OF RBC FRACTIONS OF NORMAL (AA) AND SICKLE CELL TRAIT (AS) INDIVIDUALS

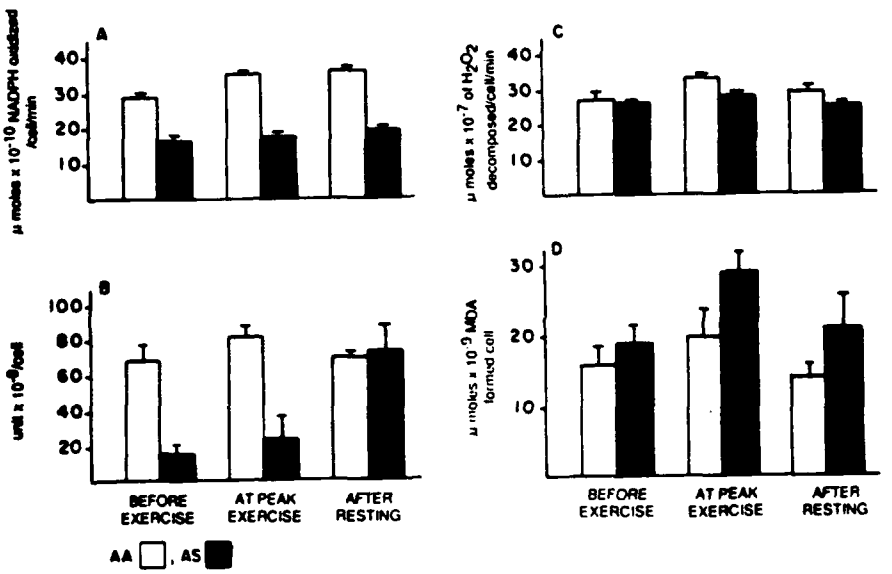

Fig. 3

EFFECT OF PHYSICAL STRESS ON (A) GLUTATHIONE PEROXIDASE, (B) SUPER OXIDE DISMUTASE, (C) CATALASE AND (D) LIPID PEROXIDATION POTENTIAL OF NORMAL (AA) AND SICKLE CELL TRAIT (AS) RBC

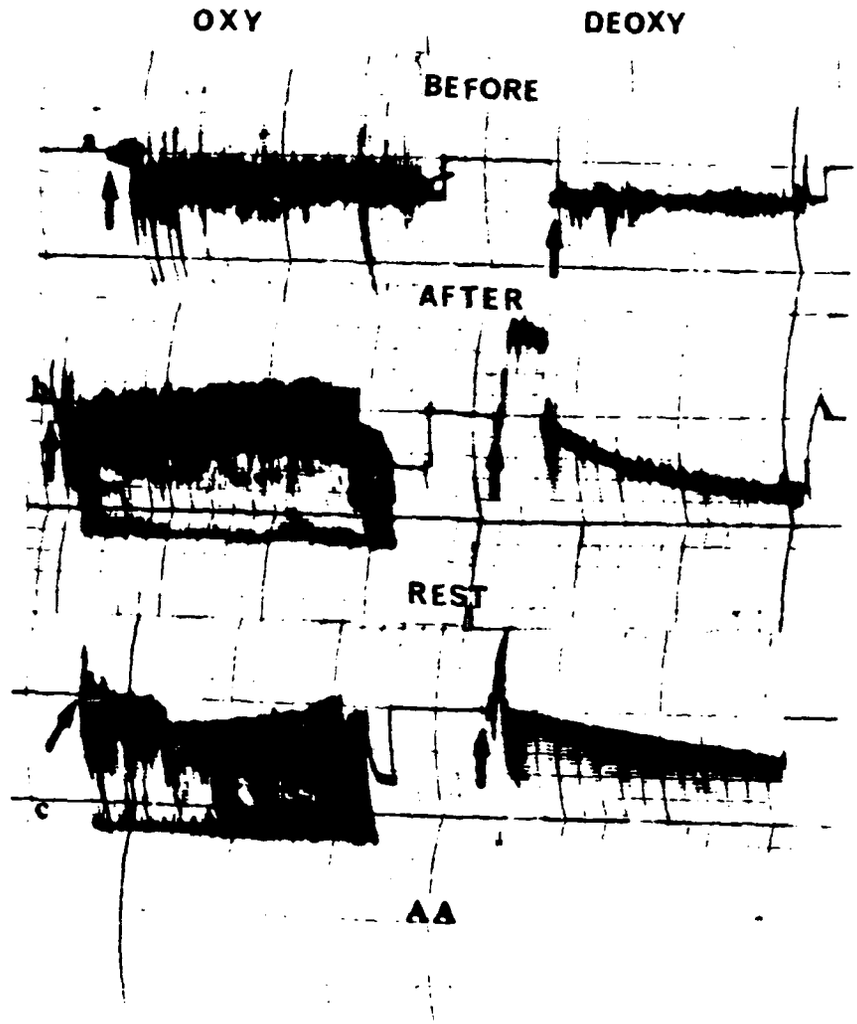

Fig. 4a

Effect of Tread Mill Exercise on Deformability of Normal RBC Under Oxygenated and Deoxygenated Conditions.

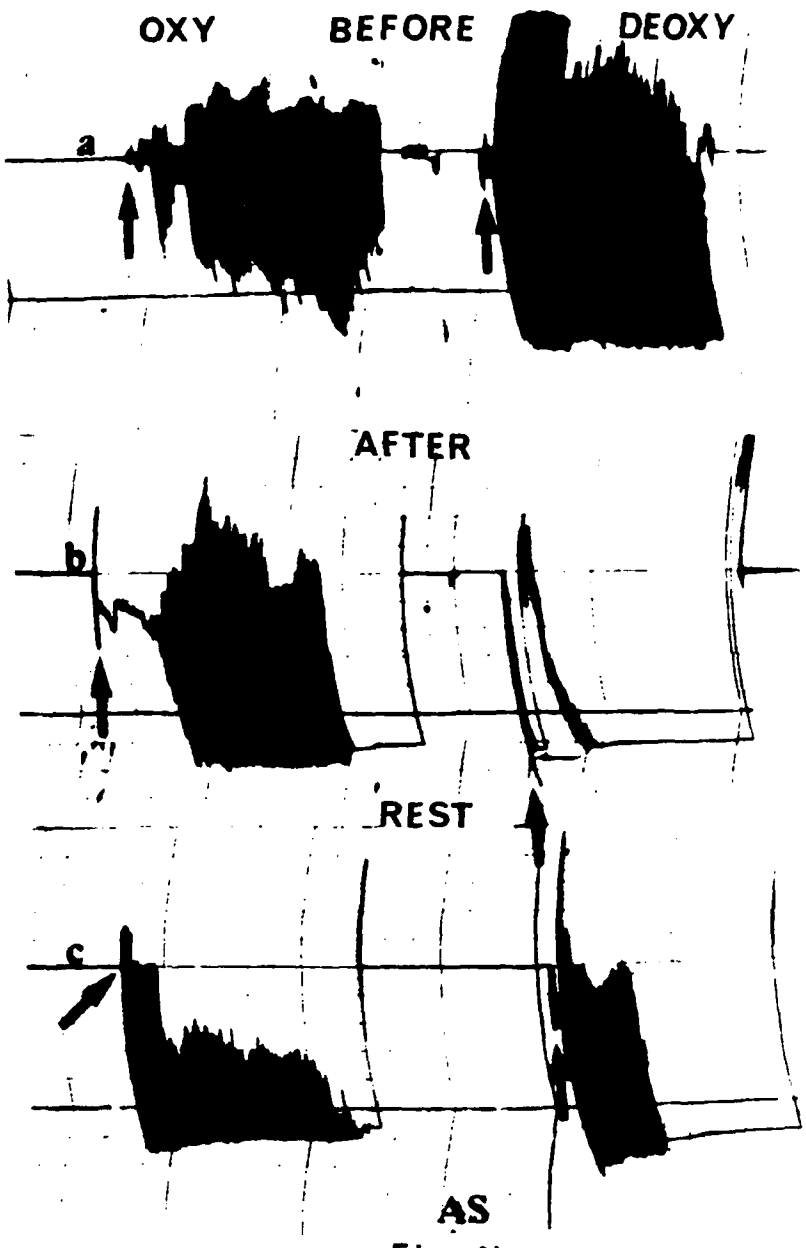

Fig. 4b

Effect of Tread Mill Exercise on Deformability of Sickle Cell Trait RBC Under Oxygenated and Deoxygenated Conditions.

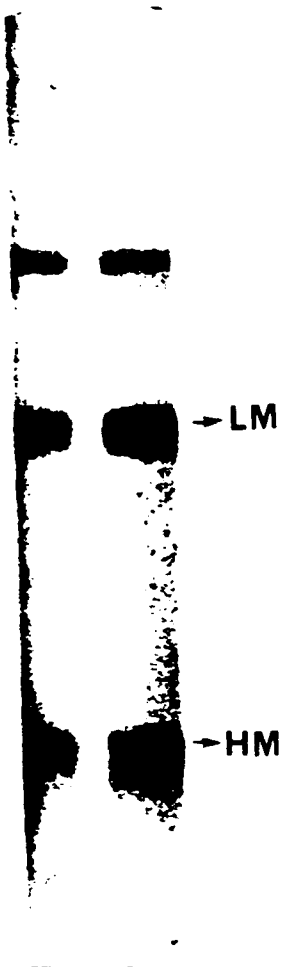

Fig. 1 Fractionation of human RBC membrane on 35-50 % linear sucrose gradient. The separation was done by centrifugation at 25,000 rpm. HM = heavy membrane; LM = light membrane.

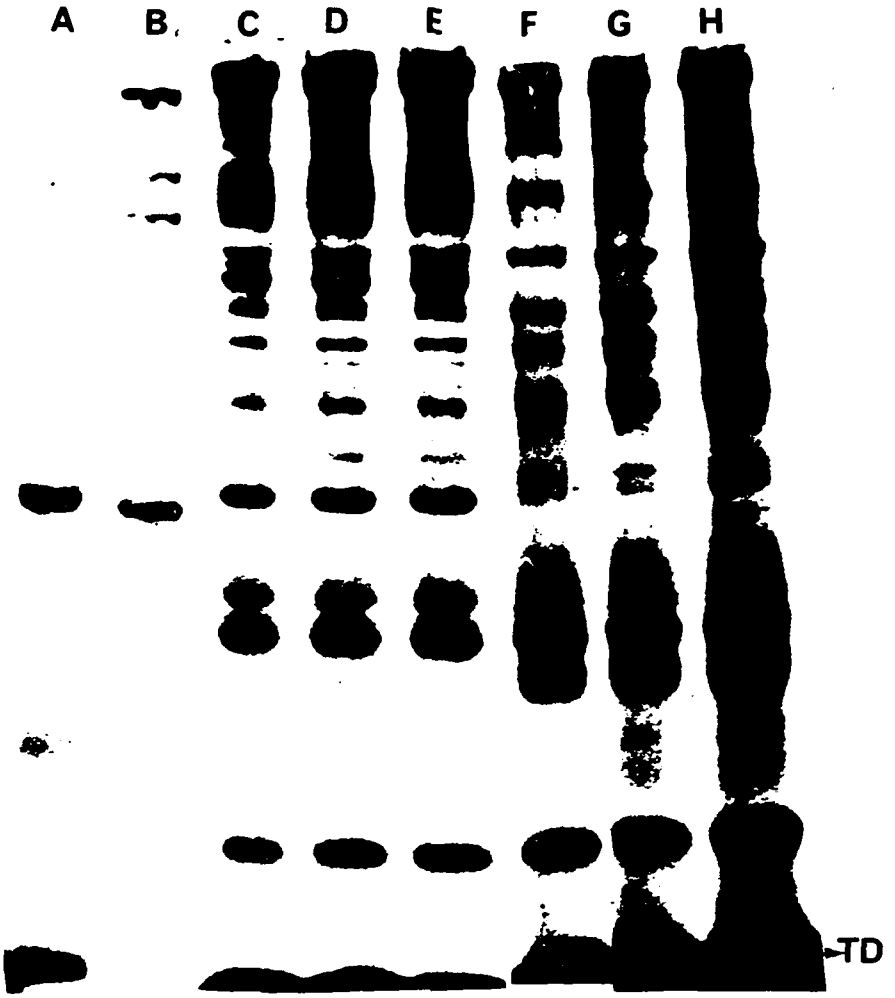

Fig.6 Electrophorogram of heavy and light membranes of human RBC in 11 % SDS-PAGE Laemmli gels. Lane A is low mol. wt. protein standards (bovine serum albumin, oval albumin, pepsin, trypsin, β lactoglobulin and lysozymes); lane B is high mol. wt. protein standards (myosin, β galactosidase, phosphorylase b, bovine serum albumin and oval albumin); Lanes C, D and E represent light membranes and lanes F, G and H represent heavy membranes. TD represents the tracking dye bromophenol blue. The electrophorograms were stained with both Ag and coomassie blue.

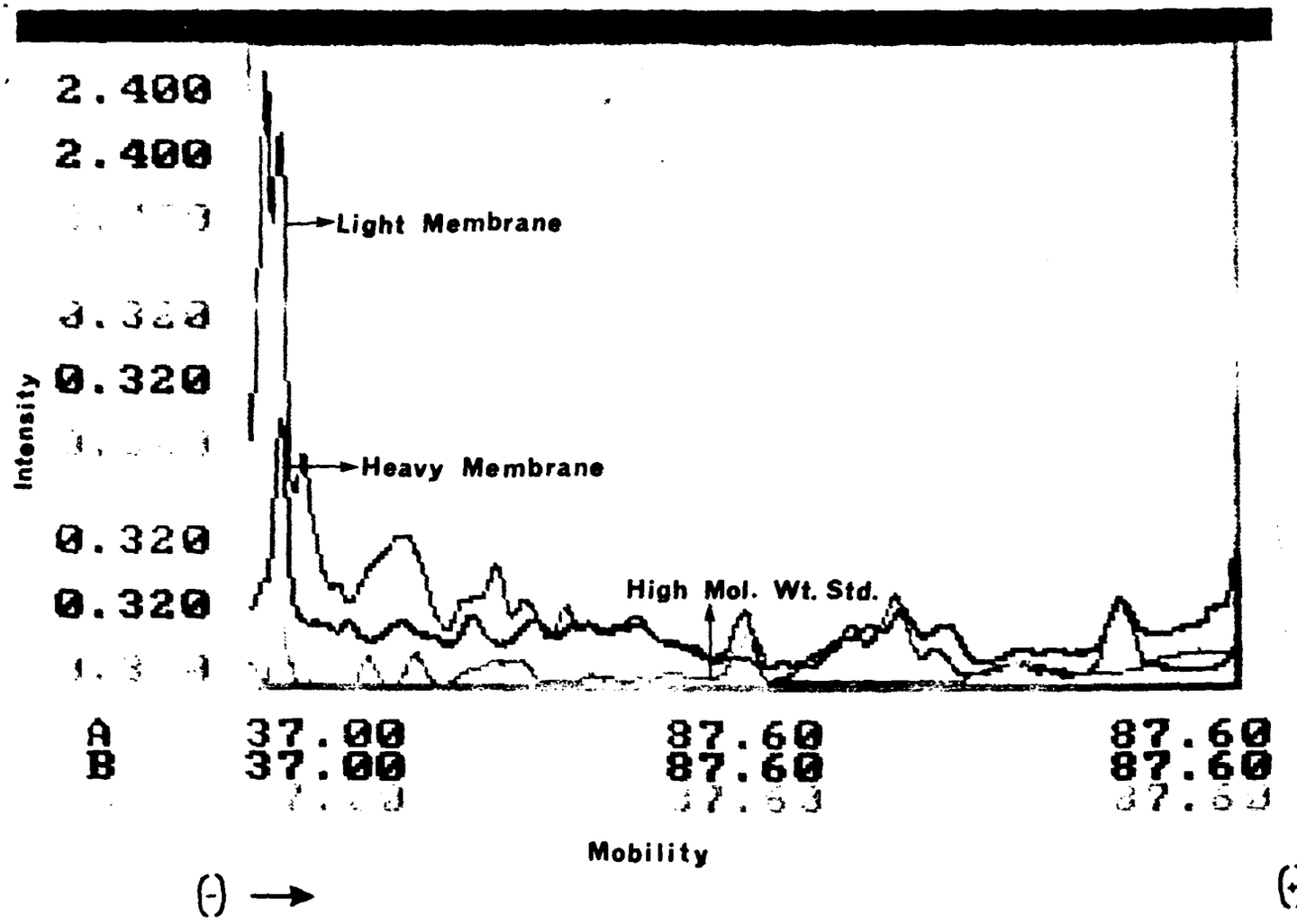

Fig. 7 Scanning of SDS polyacrylamide electrophoresis gels on a LKB 2222-010 UltroScan XL Laser Densitometer.

LIST OF PUBLICATIONS

1. Das, S.K. (1987) Lipid Peroxidation in Sickle Cell Trait Red Blood Cell Membrane, Sickle Cell Trait In - Process Review, United States Army Medical Research and Development Command, Fort Detrick, Maryland, September 21-22.

2. Mukherjee, S., Hinds, J.E., Olson, E.J. Weaver, C.G., Childs, K.R., Khan, Q.A., Hardy, R.E. and Das, S.K. (1988) The Effect of Physical Stress on Calcium and Peroxide Scavengers in Normal and Sickle Cell trait RBC. FASEB journal, Vol. 2, No. 5, A 1224.

3. Mukherjee, S. and Das, S.K. (1989) Heterogeneity of Human Red Blood Cell Membrane: Co-existence of Heavy and Light Membranes, The Journal of Cell Biology, Vol 107, No. 6, part 3, p. 357, joint ASCB/ASBMB meeting, San Francisco, Calf., Jan. 29 - Feb. 2, 1989.

4. Das, S.K., Hins, J.E., Ghosh, S. and Mukherjee, S. (1989) Effect of Physical Stress on Peroxide Scavengers, Lipid Peroxidation and Deformability in Sickle cell Trait RBC. Annual Meeting of the Comprehensive Sickle Cell Disease Centers, Durham, N.C., April 16 -18.

5. Mukherjee, S., Olson, E., Hall, L.C., Hinds, J.E. and Das, S.K. (1989) Effects of Physical Stress On Membrane Bound ATpases, Calcium and Deformability in Sickle cell Trait RBC. Annual Meeting of the Comprehensive Sickle Cell Disease Centers, Durham, N.C., April 16 -18.

6. Mukherjee, S. Hinds, J.E., Hardy, R.E. and DAs, S.K. (1989) change in Peroxide Scavengers and loss of deformability of Sickle Cell Trait (SCT) RBC in Physical Stress, 14th Internat. Cong. Of Nutr., Aug. 20 - 25.

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